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STUDIES ON THE GONOCOCCUS.* I.

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The present study is based on the study of 15 strains of *Micrococcus gonorrhea* (Neisser) obtained from the urethral discharges of acute cases of gonorrhea in men. All primary cultures were made on ascites fluid agar or on rabbit blood agar.

CULTURE MEDIA.

Body fluid media.—For the successful cultivation of the gonococcus slightly acid (+0.7 to 1.5) media, containing preferably human body fluids, such as ascitic or hydrocele fluid, blood or blood serum, are essential. I have been unable to substantiate the claims of Thallman,[1] Paldrock, and others as to the utility of simple media, urinary and otherwise. They are of no value whatever. Hydrocele or ascitic broth is of considerable value in the detection of contaminating organisms and in the preservation of cultures of gonococci for considerable periods of time. Contrary to the statements of some observers, the gonococci grow only on the surface of this medium. The sediment consists wholly of dead organisms and their debris. Blood agar, prepared both by admixture of blood before the agar solidifies and by smearing blood over the solid agar surface, is valuable, but less so than the ascitic or hydrocele agar. It is well to emphasize the importance of a slightly acid reaction in all media. It has been pointed out that human blood serum, hydrocele, and ascitic fluids may be alkaline to litmus and acid to phenolphthalein. In general, an acidity of +0.7 to 1.5 (10 c.c. medium requires 0.07 to 0.10 *n*/10 NaOH) will be found most satisfactory. It is only partly true that almost any alkaline medium is suitable for the growth of gonococci. Occasionally, abundant growths may be obtained on simple alkaline

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[1] *Centralbl. f. Bakt.*, 1900, I, 27, p. 828.

media, but such growths always show excessive autolysis and precarious stability, while more often there results no growth at all.

When blood is streaked on the agar surface the individual colonies present a characteristic and unmistakable appearance. In 24 hours they are less than pinhead size, gray-white, opaque, irregularly round, slightly convex, and with a ×8 hand lens they show a crumbly consistence and a finely stellate or radiate arrangement, the red blood having settled into the radiations like red spokes in a wheel. Very minute concentric striae or lamellae may be observed with a higher power. In examining such colonies it is well to hold the tubes up to a strong light and examine the colonies from the back. The blood should not be so thick in the medium as to prevent translucence. The separate colonies are easily picked off *in toto* with a fine platinum wire. Such colonies may attain $\frac{1}{4}$-inch size in 48 hours. Old colonies take on a glistening silvery sheen.

Artificial serum medium.—The effort to find some medium which, free from body fluid, would support life in gonococci led me to try innumerable combinations, with the result that one was hit upon which promised gratifying results. This consisted of a modified Ringer's solution, the formula for which follows:

Sodium chlorid	1.08
Potassium chlorid	0.045
Calcium chlorid	0.025
Sodium bicarbonate	0.020
Agar	0.250
Nutrient broth	20.00
Distilled water	100.00

The salts and agar are dissolved in the water by the aid of heat until the fluid shows a translucent ground glass appearance. To this is added the broth and the whole is filtered while hot through gauze or cotton into test-tubes or flasks. One sterilization in the autoclave is sufficient. The medium does not stand repeated Arnold sterilization as the agar is apt to be thrown out of suspension. When cool the medium is semisolid and has a translucent silvery appearance.

The limitations of this medium are that not all strains of gonococci will grow on it, nor is it easy to prepare, nor does it lend itself readily to centrifugation. The medium will, however, support all strains of gonococci, if there be placed on its surface a few loopfuls or a tiny drop of sterile human blood. The gonococci then thrive, while contaminating cocci and bacteria of all kinds except the subtilis bacillus settle to the bottom of the tubes or flasks in trailing white, comet-like or stalactite-like festoons. The medium supports life in gonococci longer than any medium known to me, successful transplantations having been made after 100 days. It is also valuable for isolation of pure strains. It is by no means uncommon that individual gonococcus colonies on solid media contain contaminating organisms, so that pure strains are difficult to procure. Indeed, everyone who has worked long on the gonococcus knows this peculiarity and appreciates the difficulty of obtaining pure cultures. Not infrequently the procedure requires a struggle of weeks or months.

Beer-wort and media containing alcohol are of no value.

It is the observation of many bacteriologists that the gonococcus produces acid in dextrose media, but does not change saccharose, lactose, inulin, levulose, galactose, or mannite. Using ascitic broth to which the various carbohydrates were added in varying proportions and of which the reactions varied from slightly alkaline to +2.0, this predilection was confirmed. Titration of the medium after incubation at 37° C. for 14 days invariably showed increased acidity in the tubes containing glucose, but no changes with saccharose, inulin, or mannite. One strain out of 15 showed slight changes in tubes containing galactose.

The conclusions of Watabiki[1] were not confirmed, although horse serum which that author used as the diluent for his fluid media was not employed.

Media containing organic substances.—To ordinary nutrient agar and nutrient broth were added varying proportions of such animal substances as powdered mucin, fibrin, ovary, testicle, thyroid, pituitary, and lymph gland. Of media so prepared those containing ovarian and the testicular substance one to two per cent, slightly acid in reaction, proved excellent, while the others gave indifferent results. Vegetable substances, such as yeast nuclein, Irish moss, tragacanth, and acacia were used in a similar manner. Of these, acacia yielded good growths. The best combination proved to be a saturated solution of powdered acacia in nutrient agar, having a slightly acid reaction. The medium quickly becomes wrinkled, and is of no value aside from indicating a fondness of the gonococcus for the acacia enzyme. Certain animal substances containing enzymes were similarly employed, viz., pepsin, pancreatin, and ptyalin, pepsin and pancreatin yielding important and surprising results. The preparations of pancreatin used were the ordinary pancreatic substance (insoluble) and a purified pancreatin rendered soluble by acetone fractioning. Both of these preparations in one to two per cent strengths in slightly alkaline, neutral, and slightly acid nutrient agar influenced the growth of gonococci in a marked degree. In no medium was the growth so luxuriant and rapid as in the neutral pancreatic media, but the organisms at the same time appeared to undergo an extraordinarily swift autolysis. They appeared well-nigh structureless and stained very poorly with the strongest acid stains. Growth in the acid medium was somewhat slower, but still copious, and the organisms were better preserved. The growth is very short lived. Daily transplantations are necessary, and even then strains die suddenly. Growth is not invariable. Occasionally inoculations will not "take" at all, but the thick, opaque, yellowish-white, smeary, tenacious mass quickly covering the entire surface of the medium is quite characteristic.

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[1] *Jour. Med. Res.*, 1909, 20, p. 365.

Soluble pepsin in the same proportions and reactions also yielded abundant growths, but with a slightly acid reaction, quite rapid and showing much better viability and staining reaction, and less autolysis. Like pancreatin, it yielded irregular results, but one is inclined to the opinion that all irregularities in "takes" might have been due to variations in technic rather than to special properties of media or strains, since the same irregularities are to be observed with other media routinely employed. Not enough work has been done with these enzymes to warrant any conclusion but the results indicate a predilection of the gonococcus for certain enzymes and an antipathy for others. Sterile preparations of pepsin and pancreatin are difficult to obtain. Dry heat just short of 100° C. changes the color of the products although it is said not to destroy the activity of the ferments. Boiling destroys the enzyme. Good results were obtained by heating solutions of pepsin and pancreatin to 60° C. for one hour on three successive days. These substances are distinctly acid in reaction and require the addition of alkali, preferably NaOH solution, before being added to culture media. The addition of alkali also renders the substances more readily soluble. Further mention of the enzyme relationships will be made later in this paper and, it is hoped, more fully in a subsequent communication.

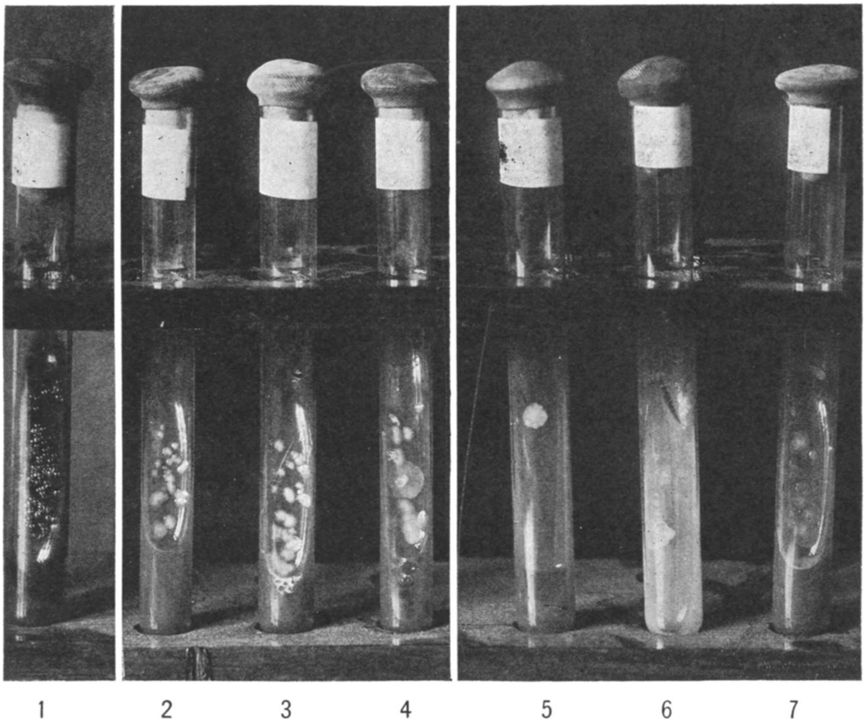

FIG. 1.

1.—Gonococcus colonies on rabbit blood agar from heart's blood of guinea-pig, five hours after intraperitoneal inoculation.

2.—Meningococcus colonies on pancreatic agar.

3.—Gonococcus colonies on pepsin agar (+1.0).

4.—Gonococcus colonies on pancreatic agar. (5 c.c. nutrient agar—0.020 solution pancreatic. Reaction+1.0.)

5.—Meningococcus colony on alkaline pancreatic agar.

6.—Gonococcus colony on alkaline pancreatic agar.

7.—Gonococcus on nutrient pancreatic agar.

Stab cultures and anaerobic culture media in general are not favorable to growth of gonococci, although unquestionably some growth does occur, provided the media contain the ascitic fluid or other favorable substance. This we should expect to be the case from our knowledge of the pathology of gonorrheal infections. The organisms survive for considerable periods of time without air. Voluminous cultures upon ascitic agar placed in a vacuum chamber over calcium chlorid and kept at 20° C. for two weeks survived and subcultures were successful.

MORPHOLOGY.

The morphology of the gonococcus is so variable as to have given rise to considerable discussion. My experience has been that by using many strains and by varying the media on which they are grown, one can observe all the types of the entire coccus family. By undoubted processes of reversion and variation it is possible, and common, to observe staphylococcus-like clumping of single forms, short chain formation as with the streptococci, lanceolate forms indistinguishable from pneumococci in shape, diplococcal forms resembling the catarrhalis groups, all in unquestionably pure cultures of gonococci. All return promptly to strain type on appropriate media. For instance, the strains grown in fluid media show the most lanceolate and chain forms; those grown on an over dry medium contain the larger number of clumps, while the cocci bred on moist ascitic agar of definite salt content and uniform reaction yield the fewest variants. From the examination of many hundreds of cultures and thousands of preparations, my opinion concurs with the view that the gonococcus is classed properly as a micrococcus. A majority of the forms are single, irregularly round and discrete. Diplococcal forms, biscuit or coffee-bean in shape, follow numerically and appear to denote active proliferation. Still a larger number of cocci in all cultures show variation and irregularity. Variations in size are common in different media. By varying the salt content of ascitic agar, for instance, one is able to breed cocci of all sizes from the small staphylococcus size to those having a diameter equal to half that of a red blood-cell. The largest cocci were produced on salt-free ascitic agar, the smallest and most stable organisms on human blood agar; among the larger cocci were many ring forms with the staining portion at the periphery and the centers shadowy.

The gonococcus is difficult to stain well. After having used a multitude of ordinary and special stains, it was found that the sharpest contours and the most brilliant staining are obtained with carbol fuchsin, one part of the ordinary tubercle carbol fuchsin and three of distilled water. Strangely enough, the methylene blue stains are very unsatisfactory for work with gonococcus cultures. The organisms do not take the stain deeply and the contours are not clear cut or sharply defined. The variations in character of different strains will embrace all variants, even for complement-fixation work.[1] My own observation would designate six selected strains as sufficient to cover all characters. The usual variations in appearance, consistence, colony forms, etc., are too well known to require further comment. The best descriptions and illustrations of cultures and organisms are by Martin.[2]

AUTOLYSIS.

It has been a matter of common observation for some time that gonococci suspended in water and in salt solution disintegrate with more or less rapidity, so that eventually no organisms whatever can be distinguished. To this peculiarity has been given the name autolysis, which is particularly apt since the property of solution or disintegration seems to be one inherent with the gonococcus. The autolysis is quite comparable to that which Rosenow and others have demonstrated in the pneumococcus. That the substance which causes the solution of the bodies of the cocci is itself a product of their life and activity is shown by the fact that autolysis occurs when all other possible causes have been removed. Gonococci which have been washed three times in distilled water, once in 0.125 NaOH solution, once in 1:5000 HCl solution, will, when again suspended in salt solution or distilled water, disintegrate with the same rapidity as unwashed cocci freshly removed from the culture medium. No culture or colony of gonococci is so young but that autolysis has already begun. Observation has shown that the life history of the individual gonococcus is, in its own sphere, comparable to the ephemeridae. Within an amazingly brief period the sequence of coccus, diplococcus, and disintegration occurs. As each organism dies it contributes a heritage which is cumulative and hastens the death of its fellows until within the space of a few days a culture of untold millions of the cocci has perished, destroyed by the combined activities of its individuals. In transplanting cultures over 48 hours old larger quantities must be transferred in order that the few live cocci therein may be included.

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[1] Torrey, *Jour. Med. Res.*, 1907, 16, p. 329, and 1908, 19, p. 471; Schwartz and McNeil, *Am. Jour. Med. Sci.*, 1911, 141, p. 693.

[2] *Jour. Path. and Bact.*, 1911, 15, p. 76.

Agents which check autolysis.—McClintock and Clark[1] have shown that a temperature of 70° C. for one hour effectually checks disintegration of suspensions of gonococci in salt solution. Temperatures less than 70° C. with or without the addition of tricresol 0.2 to 0.4 per cent do not avail.

Hydrochloric acid solution in distilled water in strength as low as 1:5000–1:10000 is the best preservative of gonococci. Suspensions of gonococci in the medium remain unchanged for months, and serve as the most stable vaccine that has so far been prepared. Tricresol may be added if desired, preferably a strength of 0.2 per cent. The gonococci are quickly killed in the solution. Organisms preserved for months in this manner resume autolysis upon washing and suspending in salt solution. Hydrochloric acid solution produces a strong agglutination of the gonococci, a fact which necessitates breaking up of the clump by shaking with glass beads, in the preparation of vaccine.

Lactic, acetic, nitric, benzoic, and oxalic acids in weak solutions in distilled water are also preservatives of the bodies of gonococci. It appears that almost any weak acid tends to overcome autolysis.

Glycerin solution five per cent in distilled water is a good preservative for short periods of time. Pepsin, 0.5 neutral, or slightly acid solution in distilled water is almost as good a preservative as dilute hydrochloric acid, but a slight yellowish-brown discoloration occurs on standing for a considerable time. Heavier pepsin percentages cause digestion.

Alcohol solutions as high as 10 per cent, weak solution of formalin, chloroform water, all sufficiently strong to prevent bacterial growth, appear to have little or no influence upon autolysis, providing the reaction of the solution is neutral or slightly alkaline. In conducting experiments on digestion one not infrequently adds some substance like chloroform, which prevents bacterial growth, but in no way hinders the activity of the digestive enzyme, and it is noteworthy that the same substances do not prevent the autolysis of gonococci.

Agents which favor autolysis.—Salt solution 0.8 to 0.9 per cent and distilled water are very favorable agents. If to either there be added *n*/10 NaOH solution or other alkali sufficient to produce slight alkalinity, it is observed that autolysis is materially hastened. Normal blood alkalinity (0.125 approximately) is a very favorable degree. It was also observed that addition of small quantities of those proteolytic enzymes which operate in an alkaline medium hastens lysis of the gonococci. In particular, it was found that the most rapid lysis of all occurred when the organisms were suspended in a slightly alkaline solution of pancreatin and preserved at 37° C. Beautiful illustrations of the disintegration may be observed in hanging-drop preparations where the process in a single field may be watched, and in stained preparations made from hour to hour.

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[1] *Jour. Infect. Dis.*, 1909, 6, p. 217.

Digestive powers of autolysates and dried gonococci.—Autolysates of gonococci were prepared by suspending 24-hour cultures in 0.9 per cent salt solution, allowing the suspension to stand at room temperature for three days, shaking with quartz sand for three hours, after which the debris was removed by passing the fluid through a Berkefeld filter. The filtrate had marked toxic effects on rabbits and guinea-pigs, three cubic centimeters being sufficient to kill a rabbit of 1,500 gms. in half an hour.

Gonococci were dried by suspending larger cultures in as small a quantity of distilled water as practicable and evaporating the fluid in a vacuum chamber. The dried substance is light brown in color. Cultures from 12 whiskey-flask surfaces yield a little over one gram of dried substance. These are also highly toxic.

With these substances the following digestive test was made:

Controls: 1. Pancreatin solution 0.020; salt solution 4 c.c.
2. Gonococcus autolysate 4 c.c.
3. Dried gonococci 0.020; salt solution 4 c.c.
4. Fibrin 0.020; salt solution 4 c.c.

Determinants: 1. Pancreatin 0.020; salt solution 4 c.c.; fibrin 0.020, alkaline to litmus.
2. Gonococcus autolysate 4 c.c.; fibrin 0.020, alkaline to litmus.
3. Gonococcus autolysate 4 c.c.; fibrin 0.020, acid to litmus.
4. Dried gonococci 0.020; salt solution 4 c.c.; fibrin 0.020, acid to litmus.
5. Dried gonococci 0.020; salt solution 4 c.c.; fibrin 0.020, alkaline to litmus.

To each tube was added a few drops of chloroform and all were placed in the incubator at 37° C. for eight hours.

Result: Controls 1, 2, 3, and 4—Negative Biuret.
Determinants 1 = +Biuret
2 = ++Biuret
3 = +Biuret
4 = ++Biuret
5 = +Biuret

It seems reasonable to assume that the gonococcus produces a proteolytic enzyme, similar to trypsin, which has a temperature range of viability between 0° C. and 70° C. It will be shown later that the physiological effects of the enzyme on laboratory animals are very strikingly similar if not identical with those of trypsin. It appears that the enzyme is capable of slight activity in an acid medium although a slight HCl acidity is capable of preventing its formation. It is a question whether this substance or enzyme can be classed with the toxins in the sense that we refer to the soluble toxin of the diphtheria bacillus and the endotoxin of the typhoid bacillus. The so-called toxins of the gonococcus, the meningococcus, and the pneumococcus, and perhaps also the streptococcus appear to form a group by themselves. Certainly those of the gonococcus and the pneumococcus present strikingly similar features.

AGGLUTINATION.

Tests of agglutination of gonococci with antisera have been disappointing in my hands. The claims of many to have produced sera which will agglutinate in dilutions of 1:50000–1:200000 I have been unable to substantiate. The most powerful serum in my series was that of a rabbit which had received three inoculations only, as follows:

February 5, 1912: Intravenously, the autolysate of one ascitic agar tube of gonococci kept for 10 days at 37° C. in two cubic centimeters of rabbit serum.

February 20, 1912: Intraperitoneally, one large loop of 24-hour ascitic agar culture of gonococci in two cubic centimeters of sterile salt solution.

February 27, 1912: Intraperitoneally, one ascitic agar tube culture suspended in two cubic centimeters sterile salt solution.

The animal was bled for serum five hours after the last inoculation.

April 20, 1912: Agglutination test.

Standard suspension of gonococci in distilled water.

Serum dilutions: 1:1 = +at once
1:1000 = +at once
1:50000 = +in one hour.

By way of comparison, a control agglutination test was made with an antigonococcus serum (017326 K. B 1095) on the same day. The results were as follows:

Dilution 1:10 = negative in two hours; partial in 18 hours.

Dilution 1:40 = negative in two hours; slight in 18 hours.

Antimeningococcus serum (018781) gave a positive agglutination of gonococci in the dilution of 1:10 in two hours.

Antidiphtheric serum, normal horse serum, and certain guinea-pig sera give positive results in eight hours' time, but almost any serum will do the same, whereas those sera produced by long-continued immunization of rabbits with large quantities of gonococci do not show that degree of agglutinative power one would expect. For instance, one rabbit received in all 16 inoculations, the first 12 intravenously at intervals of five days, the last four intraperitoneally at 10-day intervals. This serum was positive in a dilution of 1:10 in three hours and negative in a dilution of 1:40.

The substance which invariably produces the most marked agglutination of gonococci is HCl in dilution of 1:2500 and as high as 1:10000 in distilled water. Salt solution with HCl in the latter dilution is not favorable and, indeed, permits some autolysis.

Gonococcidal substances.—All ordinary germicidal substances are fatal to the gonococcus. Indeed, distilled water is destructive and salt solution even more markedly so. On the most favorable culture medium the life of the organism is very short at best. It was impossible to determine whether the silver salts, those of mercury, the acids, the alkalies, formol, the essential oils, the halogens, all in the strength usually employed clinically, were fatal, one sooner than the other, since all were quickly fatal. The experiments of Wildbolz,[1] Paldrock, and others upon the viability of the gonococcus in germicidal solutions are valuable and of great interest, but the brief periods of time, even to the fractions of minutes mentioned by some, reveal what a painstaking procedure is demanded.

ANIMAL EXPERIMENTS.

All efforts to produce a specific inflammation of the eyes or genitalia of animals were futile, as were the efforts to produce abscess, by inoculating animals with fresh cultures of gonococci.

The mechanism of defense of the mucous surfaces of all animals is much the same. The eye of the rabbit furnishes a good example. Five minutes after the conjunctival sac has been smeared with freshly isolated pure 24-hour cultures of gonococci there appears at the inner canthus a globule of grayish exudate which is seen in stained preparations to consist of mucus and immense numbers of epithelial cells, clinging to which are larger clumps of gonococci which stain poorly and appear to be undergoing rapid lysis. There are but few polymorphonuclear leukocytes. After 15 minutes the picture is much the same except that the cocci are much diminished in number. In 30 minutes the exudate has become more watery and consists of epithelial cells and leukocytes, but evidence of phagocytosis is wanting. In an hour, all cocci have disappeared and the eye appears normal. At no time does the eye show inflammatory reaction. All efforts to hold the culture in place in the conjunctival sac by mechanical means failed. The submucous injections of cultures were also negative.

The few positive results obtained in animals will be briefly outlined.

Monkeys (M. rhesus) small; 7 pounds weight.—No. 1. Hypodermic injection of morphin sulphate 2 grains; intradural injection of one-half tube of ascitic agar 24-hour culture suspended in 2 c.c. normal monkey serum. Elevation of temperature (104°.5 F.), coma, convulsions, and death in nine hours. Autopsy showed local fibrin-like exudate at site of inoculation and reddening of the meninges. Cultures negative. Exudate showed a few poorly stained shadowy cocci, leukocytes, and debris; no organisms.

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[1] *Arch. f. Dermat. u. Syph.*, 1903, 64, p. 471.

In the left conjunctival sac of the same monkey there was placed one-half tube of ascitic agar tube culture 24 hours old on the end of a spud synchronously with the intradural injection. Postmortem appearances: right eye normal; left eye protruded, lids everted and swollen, conjunctiva chemotic. Dirty gray exudate in moderate amount containing leukocytes, epithelial cells, and debris. Smears and cultures negative for gonococci. Sections of lower lid and conjunctiva showed inflammation and edema but no organisms.

No. 2. Morphin sulphate 2 grains. Intravenous injection of one-half tube of ascitic agar tube culture of gonococci, 24 hours old, in two cubic centimeters salt solution. Elevation of temperature (106° F.), coma, death in 12 hours. Endocardium over chambers and valves appeared lusterless and "smoked." Smears from liver and spleen showed poorly stained, irregular, shadowy cocci. Phagocytosis absent. Cultures from heart's blood on ascitic and blood agar positive.

No. 3. (Control)—Morphin alone. Recovered.

No. 4. (Control)—Intravenously one-half ascitic agar tube culture in two cubic centimeters salt solution. Temperature maximum 104° F. Recovered.

No. 5. (Control)—Intraperitoneally, one-half ascitic agar tube culture in two cubic centimeters salt solution. Recovered.

Rabbits.—It was demonstrated that phagocytosis plays small part in the immunity of these animals, as well as in other laboratory animals. This statement is based on a large number of examinations of blood and peritoneal exudates in inoculated animals, and in many phagocytic experiments *in vitro* with the blood of animals.

Rabbits react to both intravenous and intraperitoneal injections of gonococci, with prompt rise of temperature, which quickly returns to normal.

Guinea-pigs.—Guinea-pigs of all laboratory animals manifest the nearest approach to a true septicemia following inoculations with cultures of gonococci. The response is prompt and definite and in every instance where a fatal dose was given, gonococci were recovered in cultures from the heart's blood and the organisms were demonstrable in smears from the liver, spleen, and lungs. Phagocytosis is not a conspicuous feature, although it appears to be somewhat more common in this species than in others. The initial fatal dose of most strains is the 24-hour growth of one ascitic or blood agar tube. This was suspended in two cubic centimeters of sterile salt solution and injected into the peritoneal cavity. The animal is at once ill, and the temperature begins to fall and continues to fall steadily until the animal dies in from five to 12 hours. Animals surviving longer than 12 hours usually recover. The peritoneal exudate after one hour is serous and tinged with blood. It contains numerous red cells, innumerable well staining gonococci, and only few leukocytes of any kind. The few polymorphonuclear cells observed seldom contain cocci. After three or four hours, and after death, the fluid exudate, about 3 to 5 c.c. in amount, is grayish and contains flakes of lymph. The peritoneum is flecked with lymph, particularly about the liver and over the intestine. At this time the exudate contains greater abundance of leukocytes, but still the polymorphonuclear cells are few and phagocytosis is not conspicuous. Those cocci which live within the cells are ragged, misshapen, and do not take the stain well. Gonococci are still numerous but are rapidly diminishing in numbers and losing their outlines. The liver and spleen are usually dark red, enlarged, and congested. Cocci are always demonstrable in smears from the pulp of these organs, being for the most part free while a few poorly stained organisms are seen within the leukocytes. Cultures from the heart's blood are almost invariably positive, and from this fact it was learned that the quickest and surest method of purifying contaminated strains of gonococci is by injecting the culture in large quantities into guinea-pigs intraperitoneally, and inoculating the heart's blood on blood agar five hours later, after chloroform death.

The strain of gonococci resulting from such cultures was used over and over again, passing it from tube to pig and pig to tube regularly every 24 hours for 30 days. The strain then appeared to have lost some of its autolytic tendency; it took the ordinary stain better, became more hardy and adapted itself readily to ordinary nutrient agar (reaction +0.7), on which medium it survived eight days at 37° C. and was carried on through 22 subcultures. The impression was gained that the gonococcus is capable of modification by environment into an organism of fair stability, in spite of the observation that continued operation of the same strain through guinea-pigs did not appear to lessen the quantity required for a lethal dose.